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Army Medical Research Lab., Fort Knox, Ky. (Report No. 82)

Protective Effect of Serotonin and of Para-Aminopropiophenone Against Lethal Doses of X-Radiation - AMRL Project No. 6-59-08-013

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FORT KNOX, KENTUCKY

REPORT NO. 82
12 May 1952

PROTECTIVE EFFECT OF SEROTONIN AND OF PARA-AMINOPROPIOPHENONE AGAINST LETHAL DOSES OF X-RADIATION*

*Subtask under Effects of Irradiation, AMRL Project No. 6-59-08-013, Subtask, Enzyme, Endocrine and Metabolism Studies in Total Body Irradiation.

MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

REPORT NO. 82

PROTECTIVE EFFECT OF SEROTONIN AND OF PARA-AMINOPROPIOPHENONE AGAINST LETHAL DOSES OF X-RADIATION*

by

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Fort Knox, Kentucky
12 May 1952

*Subtask under Effects of Irradiation, AMRL Project No. 6-59-08-013, Subtask, Enzyme, Endocrine and Metabolism Studies in Total Body Irradiation.

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AMRL Project No. 6-59-08-013
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MEDEA

ABSTRACT

PROTECTIVE EFFECT OF SEROTONIN AND OF PARA-AMINOPROPIOPHENONE AGAINST LETHAL DOSES OF X-RADIATION

OBJECT

To investigate the correlation between tissue oxygen tension and radiation sickness. To study the possible protective effect of serotonin and the effect of methemoglobin-producing substances against lethal doses of x-radiation.

RESULTS AND CONCLUSIONS

Rats were subjected to a dosage of 880 r giving a 28-day survival rate of 10-14%. Serotonin (5-hydroxytryptamine) at a dose level of 4 mg/kg body weight, injected 5 minutes before exposure produced little, if any, protection. However, 20 mg/kg administered at the same interval prior to exposure produced a striking protective effect, as indicated by a survival rate of 97%.

Pre-treatment with para-aminopropiophenone in doses of 32 and 16 mg/kg 30 minutes before x-ray exposure, producing methemoglobin levels of 70 to 78%, resulted in 28-day survival rates of 94 and 97%, respectively. Pretreatment with para-aminopropiophenone at a dose level of 6 mg/kg or with sodium nitrite, 60 mg/kg, producing methemoglobin levels of 52 to 56%, resulted in 28-day survival rates of 47 and 30%, respectively.

The protective effect elicited by serotonin (20 mg/kg) is assumed to result from the vasoconstrictor property of this agent causing a transient tissue anoxia in a manner similar to that of epinephrine.

The protective effect of methemoglobinemia at certain levels is probably caused by a decreased supply of oxygen to the tissues which renders them anoxic.

The findings on the protective action of serotonin and para-aminopropiophenone against radiation seem to substantiate the concept of a relationship between tissue oxygen tension and radiosensitivity.

RECOMMENDATIONS

None.

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PROTECTIVE EFFECT OF SEROTONIN AND OF PARA-AMINOPROPIOPHENONE AGAINST LETHAL DOSES OF X-RADIATION

I. INTRODUCTION

In a previous publication (1) it was demonstrated in rats that pre-treatment with pitressin or epinephrine afforded pronounced protection against total body x-irradiation. The protective effect was assumed to result from the production of a temporary tissue anoxia by these agents. Further investigation of this pre-protection by vasoconstrictor agents was made employing serotonin (5-hydroxytryptamine), the vasoconstrictor agent present in blood platelets (2). In addition, to substantiate further the concept of the possible significance of the amount of oxygen in the tissue with regard to radiation effects, studies have been made on methemoglobinemia as a means of producing a reduced tissue oxygen tension.

II. EXPERIMENTAL

Male Sprague-Dawley rats, weighing 270 ± 10 grams, were irradiated in pairs, one serving as control, the other as a treated animal. Each pair was exposed to total body x-irradiation for 22 minutes in a single exposure. Radiation factors were: 200 kv, 6 ma, 1/2 mm Cu 1 mm Al filter, target distance approximately 29 cm, and 40 r/min, dosage rate measured in air*.

Either 1 or 5 mg of serotonin creatinine sulfate** in 1/2 ml of water were injected intraperitoneally 5 minutes before exposure. Methemoglobinemia was produced by the administration of either sodium nitrate or para-aminopropiophenone. Sodium nitrate was dissolved in isotonic saline to a concentration of 20 mg $NaNO_2$ per ml. Doses of 0.75 ml

* The authors wish to express their appreciation to the Radiobiology Dept. of this laboratory for assistance in the irradiation procedure.

** We are grateful to the Abbott Laboratories, Chicago, Illinois, and to the Upjohn Company, Kalamazoo, Michigan, for supplying us with samples of this preparation.

were administered intraperitoneally 10 or 30 minutes before exposure Dosage levels of para-aminopropiophenone* were 8, 4 and 1. 5 mg per animal, each administered intraperitoneally in 1 ml of propylene glycol. Blood methemoglobin levels were determined by a modification of the method of Evelyn and Malloy, as described by Storer and Coon (3). All controls received equivalent amounts of the appropriate solvent. Animals were housed in individual cages and weighed daily until death or termination of the experiment after 28 days.

III. RESULTS

As would be expected with an 880 r dose in total body exposure, the majority of deaths occurred between 6 and 14 days. The effect of serotonin creatinine sulfate on survival rate after total body x-irradiation is shown in Table 1. A dose level of 4 mg/kg body weight injected 5 minutes before exposure produced little, if any, protection; however, 20 mg/kg administered at the same interval prior to exposure produced a striking protective effect, as indicated by a survival rate of 97%, as compared with 6% survival for the control group.

Sodium nitrite and para-aminopropiophenone (PAPP) in varying doses were used to produce different blood levels of methemoglobin.

Figure 1 shows the per cent methemoglobin formed over a period of 90 minutes following injection of sodium nitrite or PAPP. With PAPP, dosage levels of 32 and 16 mg/kg body weight produced methemoglobin levels of 70 to 78% during the period (shown by the shaded area) that irradiated animals were exposed. Sixty mg/kg body weight of $NaNO_2$ produced a methemoglobin level of 52 to 56%, comparable to that elicited by a dosage of 6 mg/kg of PAPP. The survival rates obtained with these doses of PAPP and $NaNO_2$ are shown in Table 1. The two higher doses of PAPP, 32 and 16 mg/kg, gave excellent protection as evidenced by the 94 and 97% survival at 28 days. $NaNO_2$ (60 mg/kg) and PAPP (6 mg/kg) administered 30 minutes before exposure gave very nearly

* We are grateful to the Dow Chemical Company, Midland, Michigan for supplying us with this preparation.

the same slight protection when the 28-day survival rates were compared with their respective controls. One group injected with $NaNO_2$ (60 mg/kg) 10 minutes before x-irradiation showed no protective effect with an average methemoglobin level of 42% during the period of exposure. The control animals in the PAPP experiments received 1 ml of propylene glycol 30 minutes prior to exposure and the survival rate in this group was higher than in the untreated or saline treated controls.

IV. DISCUSSION

The protective effect elicited by serotonin creatinine sulfate is assumed to lie in its vasoconstrictor property (2), producing a transient tissue anoxia in a manner similar to that of epinephrine (1). The possible influence of this agent on metabolism has not as yet been fully investigated.

Apparently there is a correlation between the degree of methemoglobinemia and protection against radiation. The protective effect of methemoglobinemia at certain concentrations may be due to the decreased supply of oxygen to the tissues, thus rendering them anoxic. The results with $NaNO_2$ and PAPP reported here agree with similar observations obtained with mice by Storer and Coon (3). The possibility that PAPP and $NaNO_2$ may affect the oxygen uptake of the tissue directly has to be taken into consideration. Cole, Bond and Fishler (4) have reported that the mortality of mice receiving 600 r or 750 r single-dose whole body x-irradiation was reduced markedly following pre-irradiation intraperitoneal injection of $NaNO_2$ (100-125 mg/kg). They discuss the possibility that nitrite protection is mediated via its effect on catalase activity. The dose level of $NaNO_2$ per kg employed by these authors is higher than that used in the present investigation. However, it was found that 60 mg/kg was very close to the lethal dose for the rats employed. Unfortunately, the above authors did not carry out any methemoglobin determinations, permitting a comparison of methemoglobin levels with the degree of protection. The observation that administration of propylene glycol apparently exerts a slight protection is in agreement with similar observations of Salerno, Mattis and Friedell (5).

The findings on the protective action of serotonin and para-aminopropiophenone, when administered prior to a lethal dose of x-radiation, seem to substantiate the concept of a relationship between tissue oxygen tension and radiosensitivity. Alterations of susceptibility to the effects of radiation by changing oxygen tension in the tissues are probably due

to the reduction in the formation of reactive decomposition products of water. In such a concept, cognizance should be taken, of course, of the effects of those decomposition products of water (such as the OH radical), which are formed on irradiation in the absence of dissolved oxygen.

In this connection, it may be opportune to refer to the observations of Bennett, Chastain, Flint, Hansen, and Lewis (6) that the life span of rats receiving 600-1400 r whole body roentgen irradiation under anoxia was considerably shortened. This occurred in spite of the protection the anoxia gave against the lethal action of x-rays in the immediate post-irradiation period of 28 days. However, the main object of the present investigation was to gain further insight into the mechanism of radiation injury and not so much to find protective means against radiation.

V. SUMMARY

The survival rate of rats, exposed to lethal x-ray dosage, was found to be significantly increased after pretreatment with serotonin creatinine sulfate (20 mg/kg) or para-aminopropiophenone (32 mg or 16 mg/kg).

The protective effect of those agents is assumed to be due to their property of producing a temporary tissue anoxia.

VI. RECOMMENDATIONS

None.

VII. BIBLIOGRAPHY

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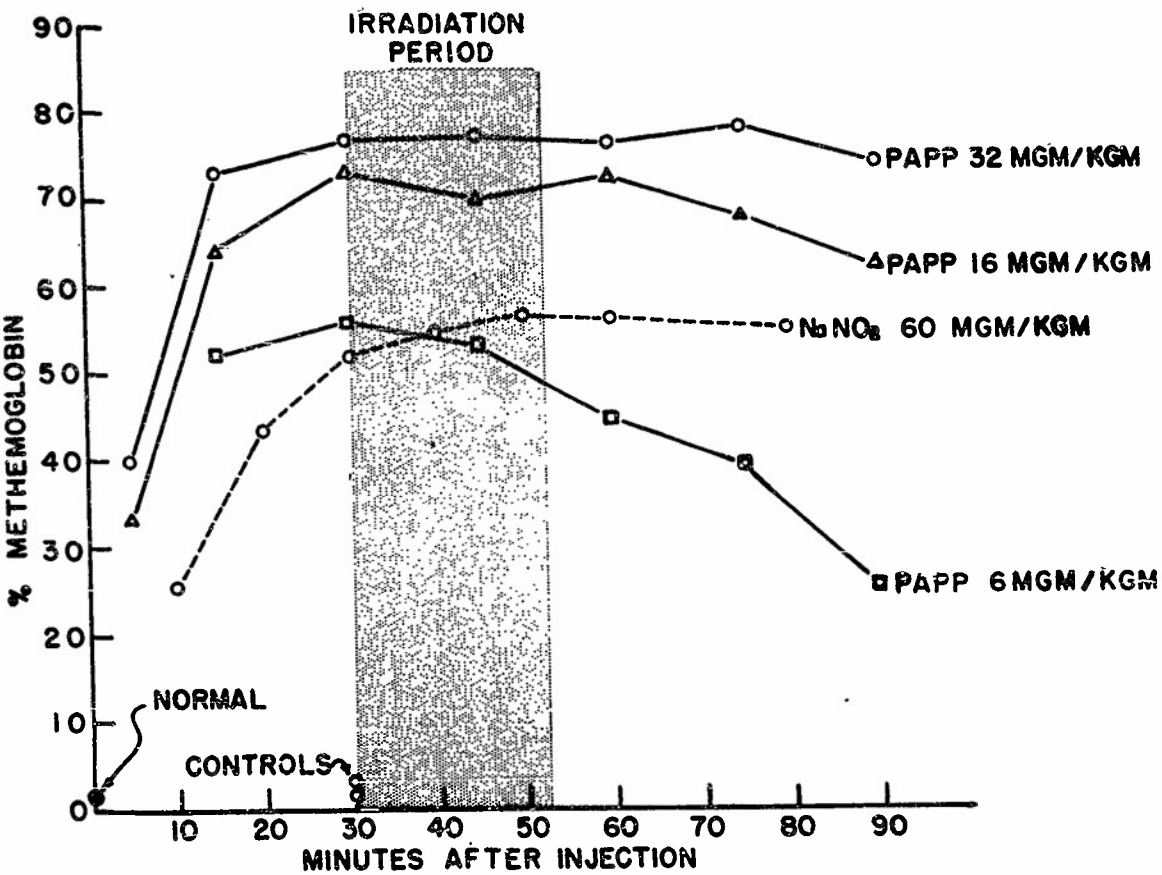
TABLE 1

EFFECT OF SEROTONIN, PAPP AND $NaNO_2$ ON
SURVIVAL OF RATS AFTER 880r TOTAL BODY X-IRRADIATION

Treatment	Time of Treatment Relative to X-Radiation	No. of Rats	SURVIVAL							
			1st Week		2nd Week		3rd Week		4th Week	
			No.	%	No.	%	No.	%	No.	%
Serotonin										
Controls (H_2O)	5 min. before	55	32	58	5	9	3	6	3	6
20 mg/kg	5 min. before	31	31	100	31	100	30	97	30	97
4 mg/kg	5 min. before	38	30	79	12	32	11	29	10	26
PAPP										
Controls (Propylene glycol)	30 min. before	77	67	87	32	42	28	36	26	34
32 mg/kg	30 min. before	31	30	97	30	97	30	97	29	94
16 mg/kg	30 min. before	30	30	100	30	100	29	97	29	97
6 mg/kg	30 min. before	30	27	90	16	53	15	50	14	47
$NaNO_2$										
Controls (Saline)	10 or 30 min. before	44	33	75	11	25	8	18	5	11
60 mg/kg	30 min. before	30	26	87	11	37	9	30	9	30
60 mg/kg	10 min. before	23	15	65	3	13	2	9	2	9



METHEMOGLOBIN LEVEL IN RATS FOLLOWING SODIUM NITRITE AND PARA-AMINOPROPIOPHENONE INJECTION.

FIG. 1